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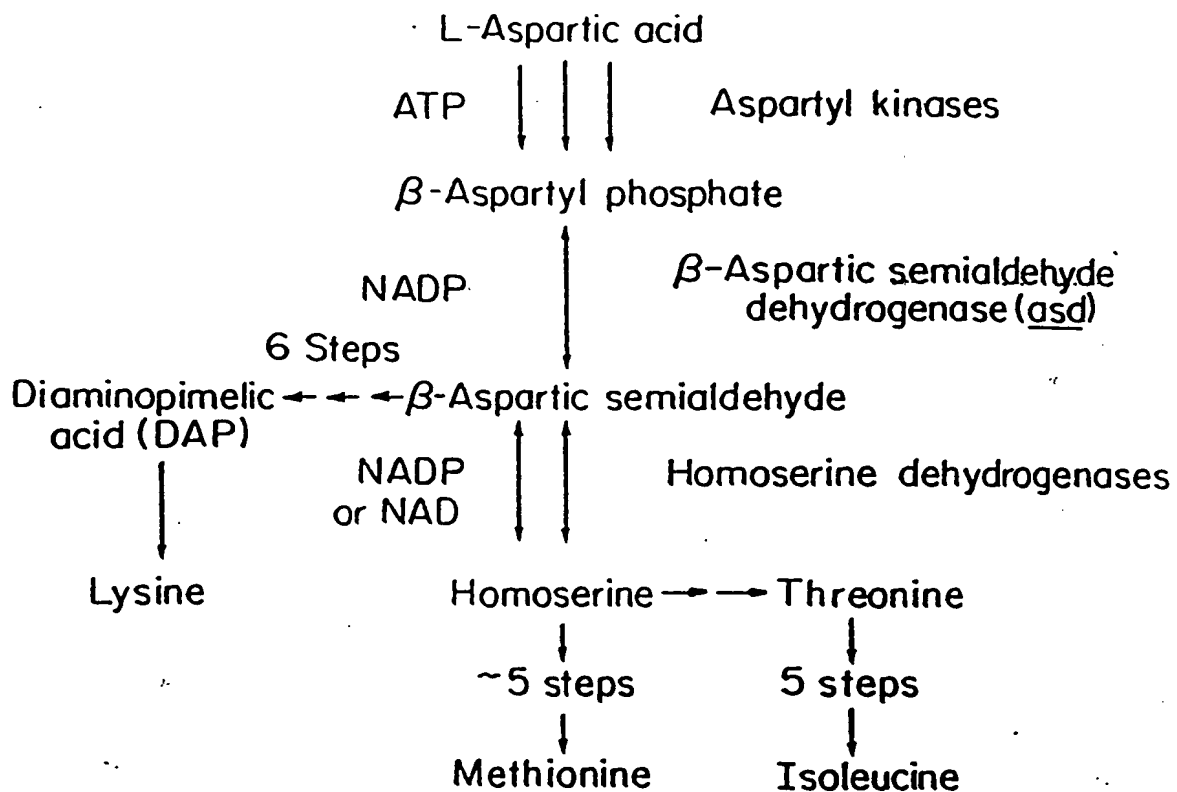


Figure 1



A

```
1 ggatcttccc taaatttaaa tataaacaac gaattatctc cttaacgtac gttttcggtc
61 cattggccct caaaccctta attaggatca ataaaacagc gacggaaatg attcccttcc
121 taacgcaaat tccctgataa tcgccactgg actttctgct tgcgcggtaa ggcaggataa
181 gtcgcattac tgatggcttc gctatcattg attaatttca cttgcgactt tggtctgttt
241 ttgtatgggt aaggatgcgc cacaggatac tggcgcgcac acacagcaca tctctttgca
301 ggaaaaaaac gctatgaaaa atgttggttt tatcggctgg cgcggaatgg tcggctctgt
361 tctcatgcaa cgcattggtag aggagcgcga ttctgacgct attcgccctg tttcttttc
421 tacctccag tttggacagg cggcgccac cttcggcgac acctccaccg gcacgtaca
481 ggacgctttt gatctggatg cgctaaaagc gctcgatata atcgtgacct gccaggcg
541 cgattatacc aacgaaattt atccaaagct gcgcgaaagc ggatggcagg gttactggat
601 tgatgcggct tctacgctgc gcatgaaaga tgatgccatt attattctcg acccggtcaa
661 ccaggacgtg attaccgacg gcctgaacaa tggcgtgaag acctttgtgg gcggttaactg
721 taccgttagc ctgatgttga tgcgctggg cggctctctt gcccataatc tcgttgactg
781 ggtatccgtc gcgacctatc aggcgcctc cggcggcggc gcgcgccata tgcgcgagct
841 gttaaccag atgggtcagt tgtatggcca tgcgccgat gaactggcga cgcgctctc
901 cgcaattctt gatattgaac gcaaagttac ggcattgacc cgcagcggcg agctgccggt
961 tgataacttt ggcgtaccgc tggcggaag cctgatcccc tggatcgaca aacagctcga
1021 taacggccag agccgcgaag agtggaaagg ccaggcgga accaacaaga ttctcaatac
1081 tgcctctgtg attccggttg atggtttgtg tgcgcgctc ggcgcgctgc gctgtcacag
1141 ccaggcggtc accatcaagc tgaaaaaaga ggtatccatt ccgacgggtg aagaactgct
1201 ggcggcacat aatccgtggg cgaaagtgtt gccgaacgat cgtgatatac ctatgcgca
1261 attaaccctg gcggcggtga ccggcacgtt gactacgccg gttggtcgtc tgcgtaagct
1321 gaacatggg ccagagttct tgcggcggtt taccgtaggc gaccagttgt tatggggcgc
1381 cgcgagccg ctgcgtcgaa tgcgcgcca gttggcgtag tggctattgc agcgttctc
1441 gggcctgctg gtggttctgt aggcgggata aggcgcgtca gcgcgccat ccggcgggga
1501 aatttggtt aaaccagggg tgcacgtca ccctttttt gcgtaataca ggagtaaagc
1561 cagatgtttc atttttatca ggagttaagc agagcattgg ctattcttta agggtagctt
1621 aatccacgg gtattaagc taacctgaag gtaggacgac gcagatagga tgcacagtgt
1681 gctgcgccgt tcaggtcaaa gaagtgtcac tacctgatgt tgaattggaa gatcc
```

B

MVKDAPQDTGAHTQHISLQEKAMKNVGFIGWRGMVGSVLMQRMVEERDFDAIRPVFFSTSQFGQA
APTFGDTSTGTLQDAFDLDALKALDIIVTCQGGDYTNIEYPKLRESGWQGYWIDAASTLRMKDDAI
IILDPVNQDVITDGLNNGVKTFVGGNCTVSLMLMSLGGLFAHNLVDWVSVATYQAASGGGARHMR
LLTQMGQLYGHVADELATPSSAILEDIERKVTAALTRSGELPVDNFGVPLAGSLIPWIDKQLDNGQSR
EEWKQAETNKILNTASVIPVDGLCVRVGALRCHSQAFTIKLKKEVSIPTVEELLAHNPNWAKVVP
NDRDITMRELTPAAVTGTLTPVGRRLRLNMGPEFLSAFTVGDQLLWGAAEPLRMLRQLA

Figure 2



Title: Functional Balanced Lethal Host Vector
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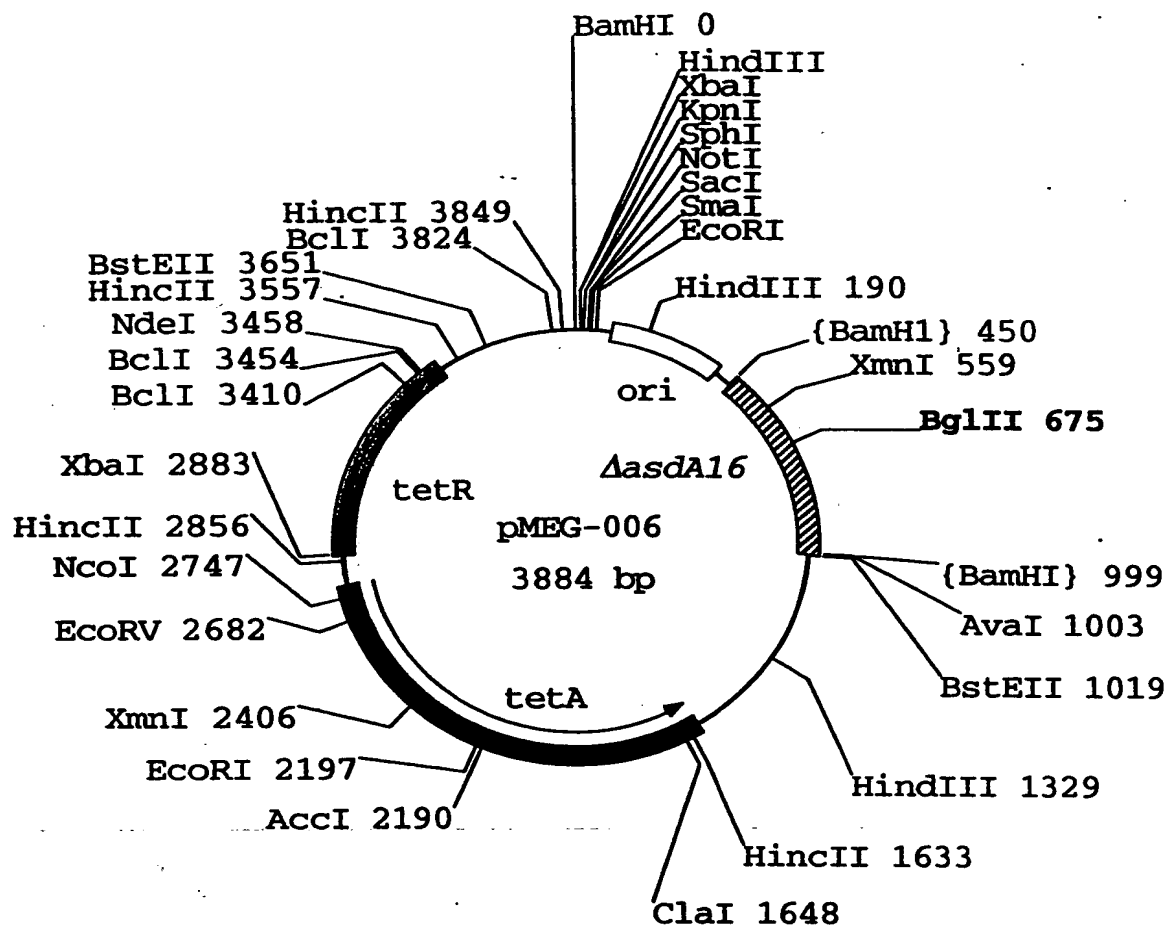


Figure 3

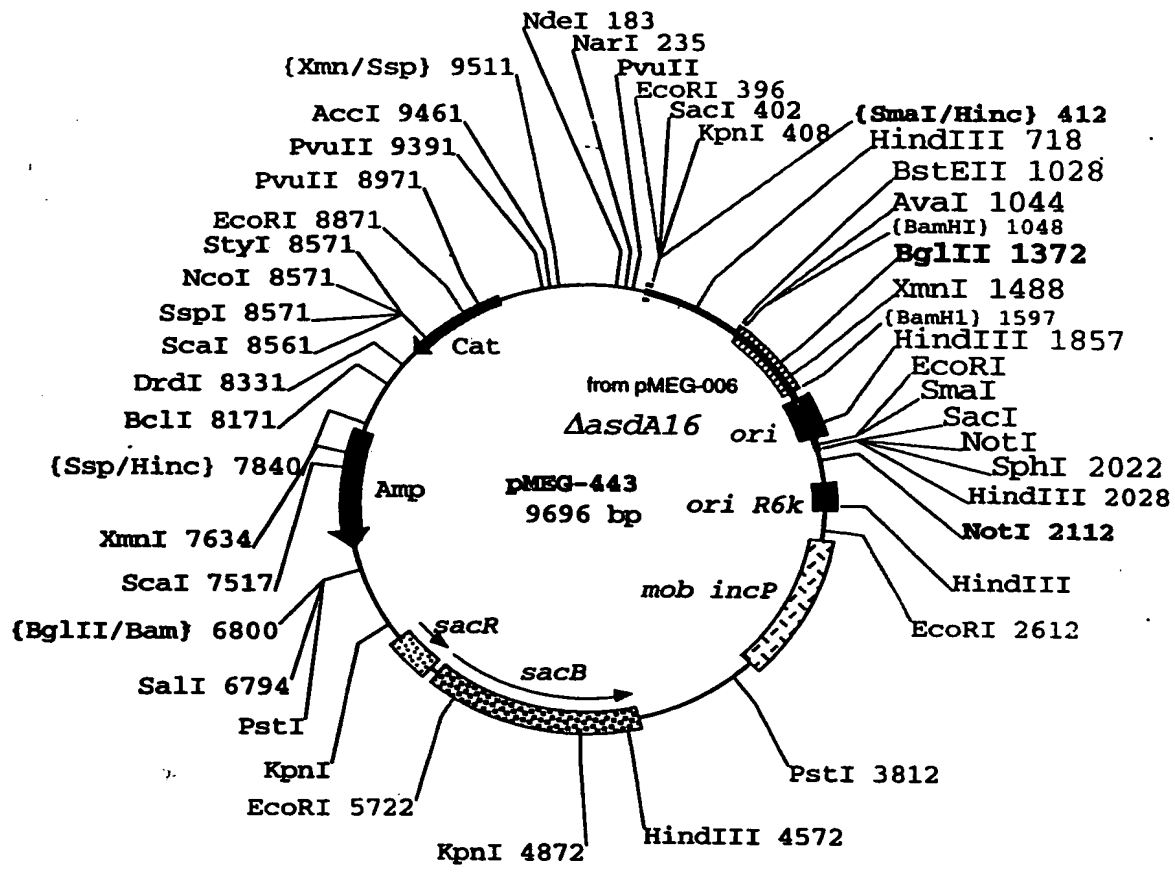


Figure 4



Title: Functional Balanced Lethal Host Vector
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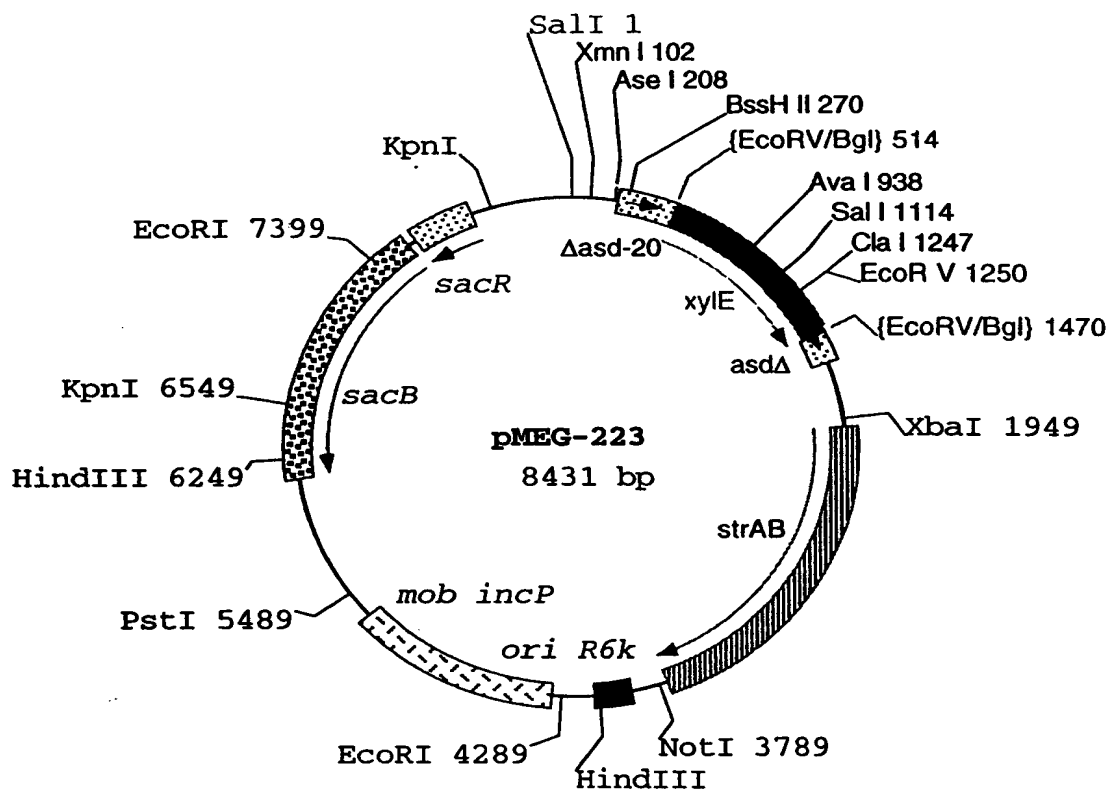


Figure 5



Title: Functional Balanced Lethal Host Vector
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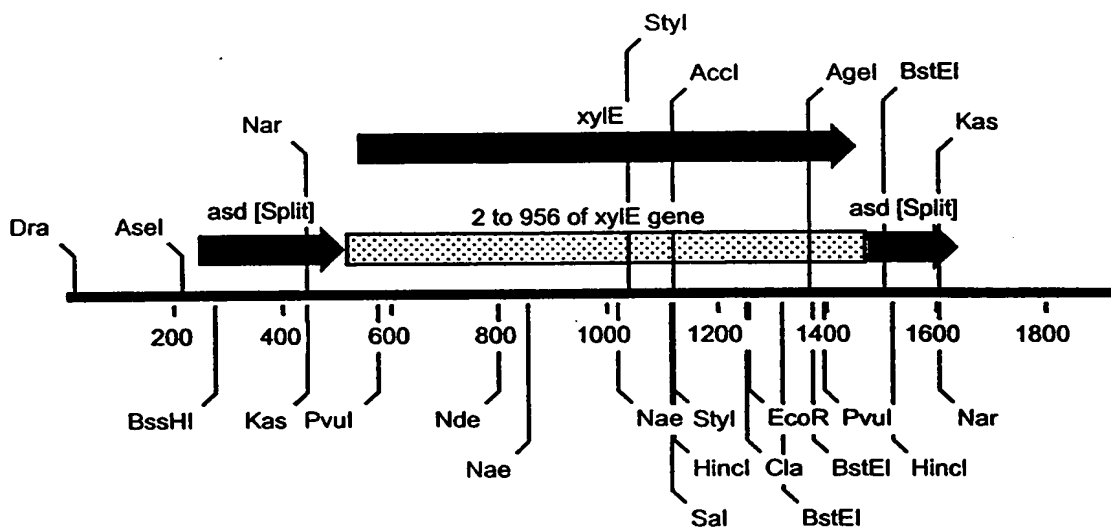


Figure 6A



Title:
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Functional Balanced Lethal Host Vector
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Figure 6B

DraI

10 20 30 40 50 60
GGATCTTCCCTAAATTTAAATATAACAACGAATTATCTCCTTAACGTACGTTTTTCGTTCC

70 80 90 100 110 120
CATTGGCCCTCAAACCCCTAATTAGGATCAATAAAACAGCGACGGAAATGATTCCCTTCC

130 140 150 160 170 180
TAACGCAAATTCCTGATAATCGCCACTGGACTTTCTGCTTGCGCGGTAAGGCAGGATAA

AseI

190 200 210 220 230 240
GTCGCATTACTGATGGCTTCGCTATCATTGATTAATTTCACTTGCGACTTTGGCTGCTTT

BssHII

250 260 270 280
TTGT ATG GTG AAG GAT GCG CCA CAG GAT ACT GGC GCG CAT ACA CAG
Met Val Lys Asp Ala Pro Gln Asp Thr Gly Ala His Thr Gln
_a_a_a_a_a_ASD SPLIT]_a_a_a_a_a_

290 300 310 320 330
CAC ATC TCT TTG CAG GAA AAA AAC GCT ATG AAA AAT GTT GGT TTT
His Ile Ser Leu Gln Glu Lys Asn Ala Met Lys Asn Val Gly Phe
_a_a_a_a_a_ASD [SPLIT]_a_a_a_a_a_

340 350 360 370
ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG
Ile Gly Trp Arg Gly Met Val Gly Ser Val Leu Met Gln Arg Met
_a_a_a_a_a_ASD [SPLIT]_a_a_a_a_a_

380 390 400 410 420
GTA GAG GAG CGC GAT TTC GAC GCT ATT CGC CCT GTT TTC TTT TCT
Val Glu Glu Arg Asp Phe Asp Ala Ile Arg Pro Val Phe Phe Ser
_a_a_a_a_a_ASD [SPLIT]_a_a_a_a_a_



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NarI
|
KasI
|

430 440 450 460
ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC
Thr Ser Gln Phe Gly Gln Ala Ala Pro Thr Phe Gly Asp Thr Ser
_a_a_a_a_a_a ASD [SPLIT] _a_a_a_a_a_a_

470 480 490 500 510
ACC GGC ACG CTA CAG GAC GCT TTT GAT CTG GAT GCG CTA AAA GCG
Thr Gly Thr Leu Gln Asp Ala Phe Asp Leu Asp Ala Leu Lys Ala
_a_a_a_a_a_a ASD [SPLIT] _a_a_a_a_a_a_

520 530 540 550 560
CTC GAT GATCTATGAAGAGGTGACGTC ATG AAC AAA GGT GTA ATG CGA CCG
Leu Asp Met Asn Lys Gly Val Met Arg Pro
a _c_c_c_XYLE_c_c_c_c_

PvuII
|

570 580 590 600
GGC CAT GTG CAG CTG CGT GTA CTG GAC ATG AGC AAG GCC CTG GAA
Gly His Val Gln Leu Arg Val Leu Asp Met Ser Lys Ala Leu Glu
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

610 620 630 640 650
CAC TAC GTC GAG TTG CTG GGC CTG ATC GAG ATG GAC CGT GAC GAC
His Tyr Val Glu Leu Leu Gly Leu Ile Glu Met Asp Arg Asp Asp
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

660 670 680 690
CAG GGC CGT GTC TAT CTG AAG GCT TGG ACC GAA GTG GAT AAG TTT
Gln Gly Arg Val Tyr Leu Lys Ala Trp Thr Glu Val Asp Lys Phe
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

700 710 720 730 740
TCC CTG GTG CTA CGC GAG GCT GAC GAG CCG GGC ATG GAT TTT ATG
Ser Leu Val Leu Arg Glu Ala Asp Glu Pro Gly Met Asp Phe Met
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

750 760 770 780
GGT TTC AAG GTT GTG GAT GAG GAT GCT CTC CGG CAA CTG GAG CGG
Gly Phe Lys Val Val Asp Glu Asp Ala Leu Arg Gln Leu Glu Arg
_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_

Figure 6B
(Con't.)



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NdeI
|

790 800 810 820 830
GAT CTG ATG GCA TAT GGC TGT GCC GTT GAG CAG CTA CCC GCA GGT
Asp Leu Met Ala Tyr Gly Cys Ala Val Glu Gln Leu Pro Ala Gly
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

NaeI
|

840 850 860 870
GAA CTG AAC AGT TGT GGC CGG CGC GTG CGT TCC AGG CCC TCC GGG
Glu Leu Asn Ser Cys Gly Arg Arg Val Arg Ser Arg Pro Ser Gly
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

880 890 900 910 920
CAT CAC TTC GAG TTG TAT GCA GAC AAG GAA TAT ACT GGA AAG TGG
His His Phe Glu Leu Tyr Ala Asp Lys Glu Tyr Thr Gly Lys Trp
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

930 940 950 960
GGT TTG AAT GAC GTC AAT CCC GAG GCA TGG CCG CGC GAT CTG AAA
Gly Leu Asn Asp Val Asn Pro Glu Ala Trp Pro Arg Asp Leu Lys
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

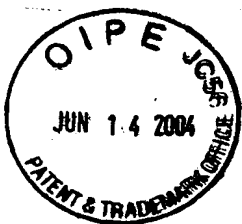
970 980 990 1000 1010
GGT ATG GCG GCT GTG CGT TTC GAC CAC GCC CTC ATG TAT GGC GAC
Gly Met Ala Ala Val Arg Phe Asp His Ala Leu Met Tyr Gly Asp
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

NaeI StyI
| |

1020 1030 1040 1050
GAA TTG CCG GCG ACC TAT GAC CTG TTC ACC AAG GTG CTC GGT TTC
Glu Leu Pro Ala Thr Tyr Asp Leu Phe Thr Lys Val Leu Gly Phe
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

1060 1070 1080 1090 1100
TAT CTG GCC GAA CAG GTG CTG GAC GAA AAT GGC ACG CGC GTC GCC
Tyr Leu Ala Glu Gln Val Leu Asp Glu Asn Gly Thr Arg Val Ala
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

Figure 6B
(Con't.)



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HincII
|
AccI
||
SalI || StyI
| |
1110 1120 1130 1140
CAG TTT CTC AGT CTG TCG ACC AAG GCC CAC GAC GTG GCC TTC ATT
Gln Phe Leu Ser Leu Ser Thr Lys Ala His Asp Val Ala Phe Ile
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

1150 1160 1170 1180 1190
CAC CAT CCG GAA AAA GGC CGC CTC CAT CAT GTG TCC TTC CAC CTC
His His Pro Glu Lys Gly Arg Leu His His Val Ser Phe His Leu
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

1200 1210 1220 1230
GAA ACC TGG GAA GAC TTG CTT CGC GCC GCC GAC CTG ATC TCC ATG
Glu Thr Trp Glu Asp Leu Leu Arg Ala Ala Asp Leu Ile Ser Met
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

EcoRV
|
ClaI
|
1240 1250 1260 1270 1280
ACC GAC ACA TCT ATC GAT ATC GGC CCA ACC CGC CAC GGC CTC ACT
Thr Asp Thr Ser Ile Asp Ile Gly Pro Thr Arg His Gly Leu Thr
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

BstEII
|
1290 1300 1310 1320
CAC GGC AAG ACC ATC TAC TTC TTC GAC CCG TCC GGT AAC CGC AAC
His Gly Lys Thr Ile Tyr Phe Phe Asp Pro Ser Gly Asn Arg Asn
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

BstEII
|
AgeI
|
1330 1340 1350 1360 1370
GAA GTG TTC TGC GGG GGA GAT TAC AAC TAC CCG GAC CAC AAA CCG
Glu Val Phe Cys Gly Gly Asp Tyr Asn Tyr Pro Asp His Lys Pro
_c_c_c_c_c_c_c_XYLE_c_c_c_c_c_c_c_

Figure 6B
(Con't.)



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PvuII
|

1380 1390 1400 1410
GTG ACC TGG ACC ACC GAC CAG CTG GGC AAA GCC TTC TTT TAC CAC
Val Thr Trp Thr Thr Asp Gln Leu Gly Lys Ala Phe Phe Tyr His
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

1420 1430 1440 1450
GAC CGC ATT CTC AAC GAA CGA TTC ATG ACC GTG CTG ACC
Asp Arg Ile Leu Asn Glu Arg Phe Met Thr Val Leu Thr
__c__c__c__c__c__c__c__XYLE__c__c__c__c__c__c__c__

BstEII
|

1460 1470 1480 1490 1500
TGATGGTCCGGAGATC ATC ACT ATG CGC GAA TTA ACC CCG GCG GCG GTG
Ile Thr Met Arg Glu Leu Thr Pro Ala Ala Val
__b__b__b__b__ASD [SPLIT]__b__b__b__b__

HincII
|

1510 1520 1530 1540 1550
ACC GGC ACG TTG ACT ACG CCG GTT GGT CGT CTG CGT AAG CTG AAC
Thr Gly Thr Leu Thr Thr Pro Val Gly Arg Leu Arg Lys Leu Asn
__b__b__b__b__b__b__ASD [SPLIT]__b__b__b__b__b__b__

1560 1570 1580 1590
ATG GGG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG
Met Gly Pro Glu Phe Leu Ser Ala Phe Thr Val Gly Asp Gln Leu
__b__b__b__b__b__b__ASD [SPLIT]__b__b__b__b__b__b__

NarI
|

KasI
||

1600 1610 1620 1630 1640
TTA TGG GGC GCC GCC GAG CCG CTG CGT CGA ATG CTG CGC CAG TTG
Leu Trp Gly Ala Ala Glu Pro Leu Arg Arg Met Leu Arg Gln Leu
__b__b__b__b__b__b__ASD [SPLIT]__b__b__b__b__b__b__

1650 1660 1670 1680
GCG TAGTGGCTATTGCAGCGCTTATCGGGCCTGCGTGTGG
Ala
—

Figure 6B
(Con't.)



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1690 1700 1710 1720 1730 1740
TTCTGTAGGCCGGATAAGGCGCGTCAGCGCCGCCATCCGGCGGGGAAATTTGTGTTAAAC
1750 1760 1770 1780 1790 1800
CAGGGGTGCATCGTCACCCTTTTTTTGCGTAATACAGGAGTAAACGCAGATGTTTCATTT
1810 1820 1830 1840 1850 1860
TTATCAGGAGTTAAGCAGAGCATTGGCTATTCTTTAAGGGTAGCTTAATCCCACGGGTAT
1870 1880 1890 1900 1910 1920
TAAGCCTAACCTGAAGGTAGGACGACGCAGATAGGATGCACAGTGTGCTGCGCCGTTTCAG
1930 1940 1950 1960
GTCAAAGAAGTGTCCTACTACCTGATGTTGAATTGGAAGATCC

Figure 6B
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Nucleotide sequences of trc promoter/operator and MCS

MCS: *NcoI* *EcoRI* ----- *HindIII*

pYA3098, pYA3148, pYA3332, pYA3333, pYA3334,
pYA3336, pYA3339, pYA3340, pYA3341, pYA3342

-35

5' ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTC

-10

GTATAATGTGTGGAATTGTGAGCGGATAACAATTTTCACAC

SD

AGGAAACAGACC *NcoI* **ATG** *EcoRI* GGC AAT TCC CGG GGA

Met Gly Ile Arg Asn Ser Arg Gly

BamHI *SalI* *PstI* *HindIII*

TCC GTC GAC CTG CAG CCA AGC TCC CAA GCT T 3'

Ser Val Asp Leu Gln Pro Ser Ser Gln Ala

Figure 7

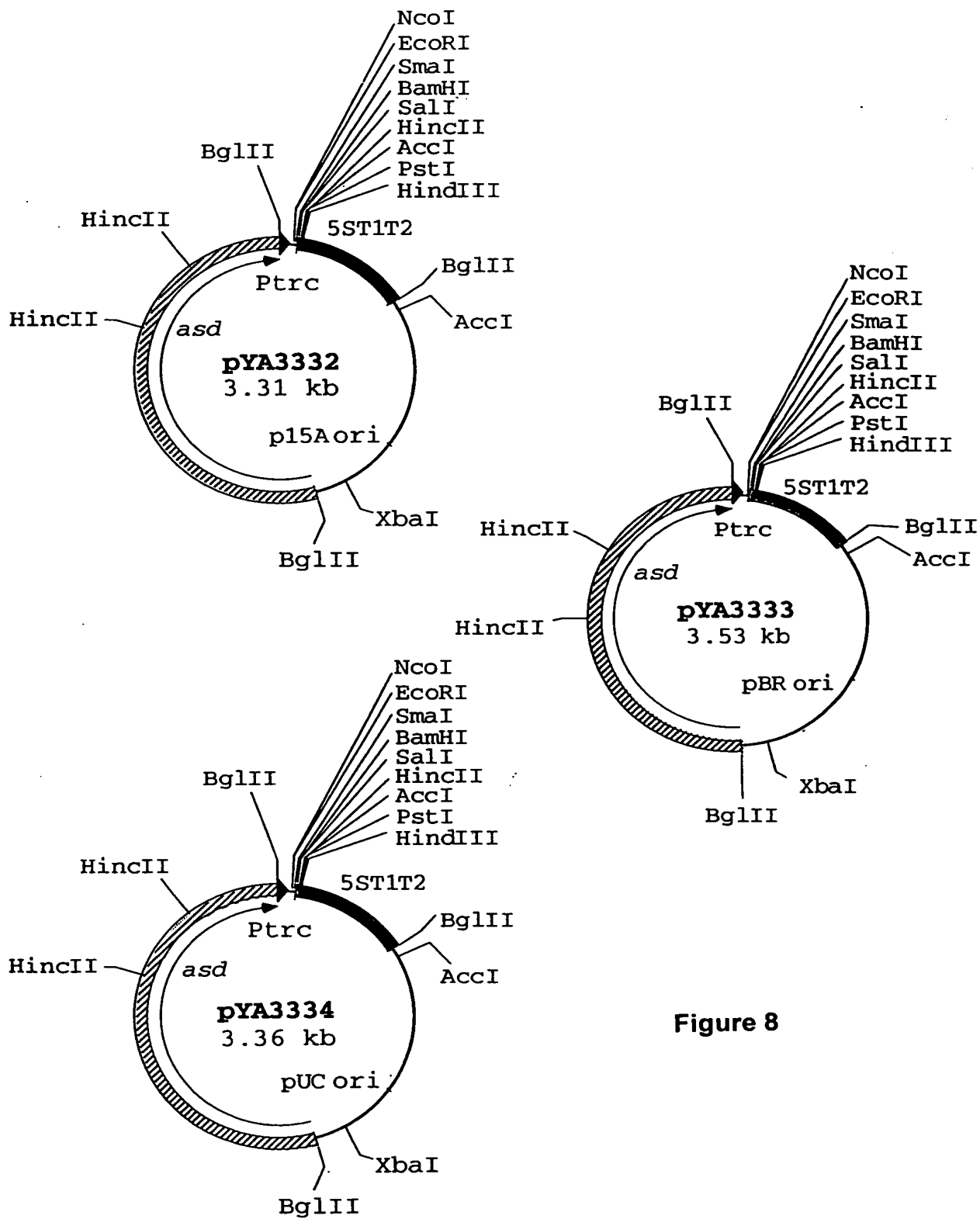


Figure 8



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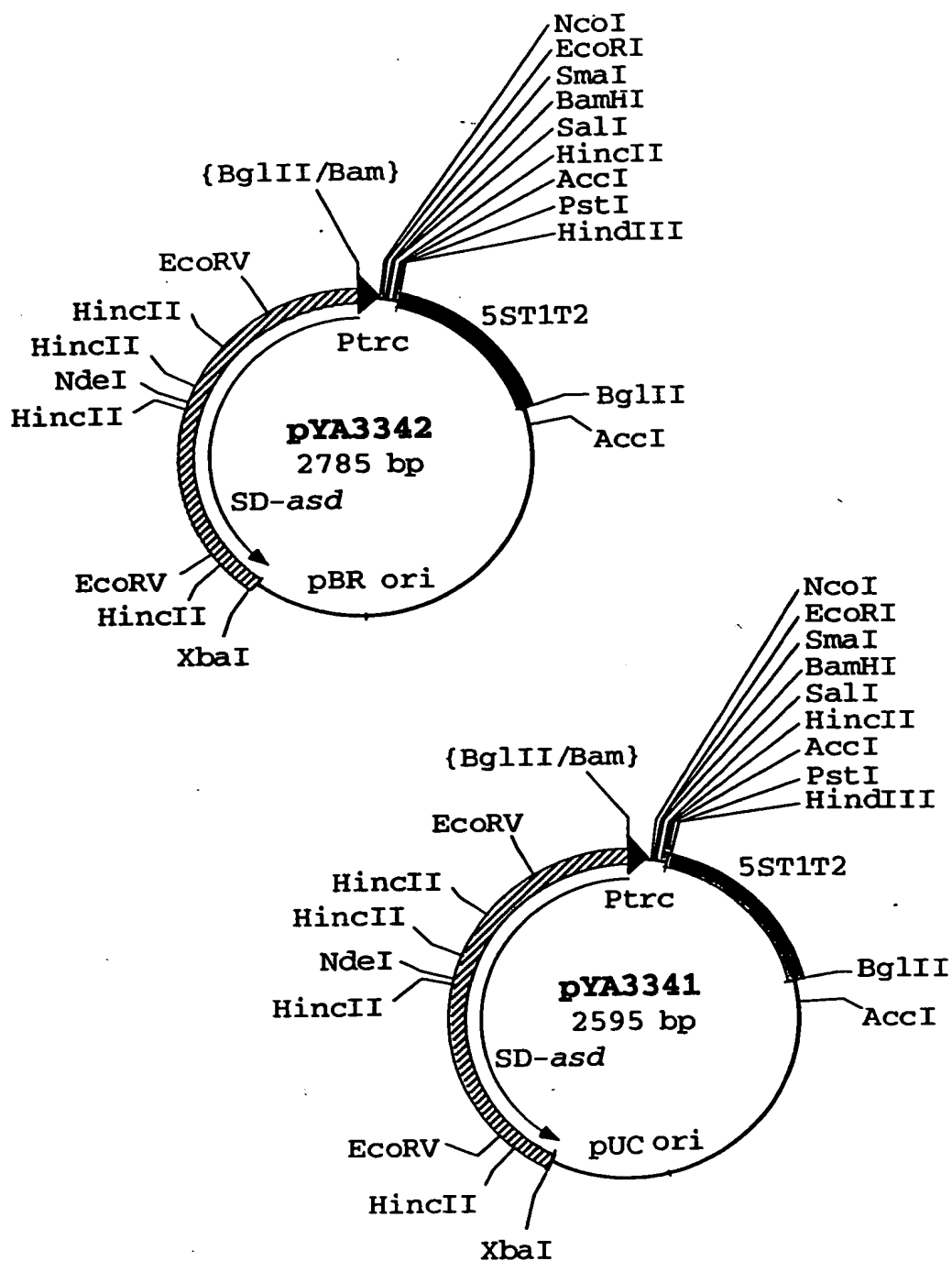


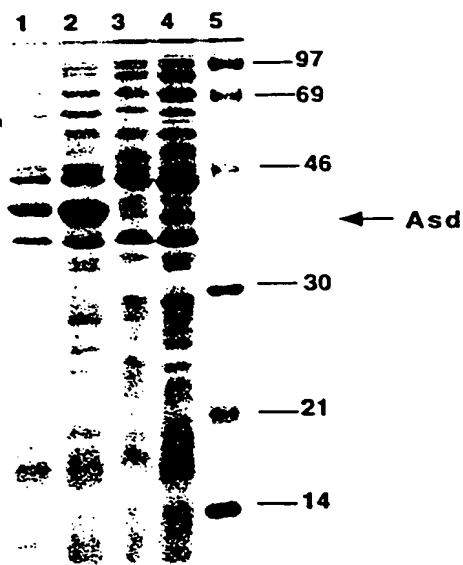
Figure 9



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**Level of Asd synthesized in recombinant
S. typhimurium strains with different Asd⁺ plasmids**



Cell lysates of *S. typhimurium* χ 4550 with pYA3333 (lane 1), pYA3334 (lane 2), pYA3342 (lane 3) and pYA3341 (lane 4). Lane 5 contains molecular weight markers. The arrow indicates Asd protein band.

Figure 10



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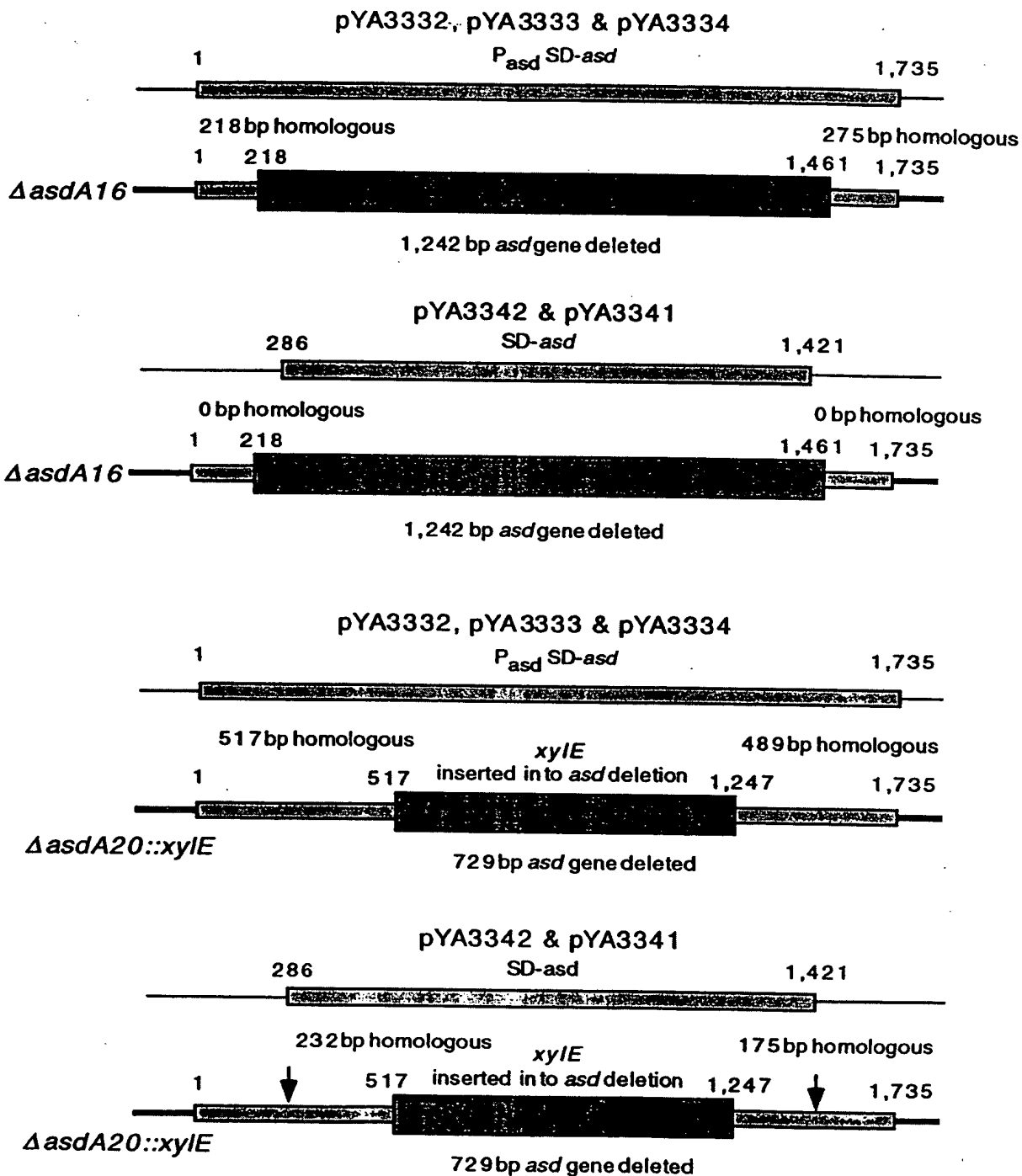


Figure 11



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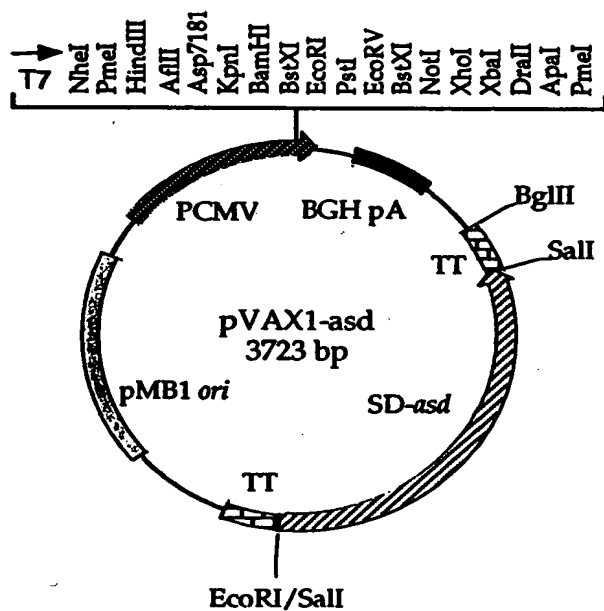


Figure 12